Conclusions: Summary of Main Trends

A wide variety of HPLC methods are available to analyse carbohydrates from various complex matrices. Aminopropylsiloxane-bonded silica of small particle diameter (3 μm), amine-bonded vinyl alcohol copolymer packings, diol- and cyclodextrin-bonded silica, as well as macroporous cross-linked vinylpyridinium polymers, are some of the columns of choice for high selectivity in the HILIC of carbohydrates. Furthermore, a large number of columns may be selected to separate carbohydrates on cation exchange resins carrying different counterions. Employing these columns the chromatographic separation is governed by a combination of size exclusion and ligand exchange mechanisms. In oligosaccharide separations, size exclusion is the primary mechanism which involves the binding of hydroxyl groups of the sugars with the fixed counterion of the resin, thus they elute last.

On the other hand, RP-HPLC with plain water as the eluent may be useful for the separation of various underivatized oligosaccharides. The mechanism governing this HPLC method produces separations that complement those given by cation exchange columns and therefore the two techniques should be used in conjunction in isolation and analysis of carbohydrates.

Column instability and low detection sensitivity are the major disadvantages using these HPLC methods. However, column and detection technologies to analyse carbohydrates are in continuous development.

Mono- and oligosaccharides are detected without any derivatization by PAD, which is sensitive and selective for detection of these analytes. The use of HPAEC is now widely accepted as a powerful analytical tool for carbohydrate analysis. However, HPAEC methods require dedicated columns and instrumentation.

See also: **II / Chromatography: Liquid:** Detectors: Evaporative Light Scattering; Detectors: Refractive Index Detectors; Instrumentation; Mechanisms: Reversed Phases. **III / Polysaccharides:** Liquid Chromatography.

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Thin-Layer (Planar) Chromatography

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Introduction

Carbohydrates are some of the more difficult compounds to separate from each other. They primarily differ in the number of carbon atoms, the configuration of the chiral centres and the size of the molecules, that is to say whether they are di-, tri- and oligosaccharides. If two carbohydrates have any one of the three characteristics in common, they can be difficult to separate. There are some carbohydrates that differ by having a special functional group, for example a carboxyl group (giving uronic, onic and aric acids),

an amino or acetyl-amino group (giving amino and *N*-acetyl amino sugars), or a phosphoric acid ester group (giving carbohydrate phosphates). Nevertheless, because carbohydrates play such an important role in living systems and comprise a large number of important naturally occurring products, methods for separating them were some of the earliest chromatographic procedures developed.

Early chromatographic methods in the 1950s for separating carbohydrates used paper as the solid support. In the early 1960s Thoma introduced thin-layer chromatography (TLC) for separating carbohydrates. The use of TLC, however, was not particularly easy and convenient at that time, as each laboratory had to prepare its own thin-layer plates. This led to a wide variation in the quality of the plates and in the results that were obtained, even within a single laboratory. The results were difficult to reproduce from day to day and particularly from laboratory to laboratory. It was not until reproducible thin-layer plates were commercially developed and produced at a reasonable price, primarily by Whatman and Merck in the late 1970s, that TLC became a viable and valuable procedure.

TLC has now become the principal method of choice for the separation of carbohydrates. High performance liquid chromatography (HPLC) has displaced TLC in many laboratories, although for carbohydrates and other materials, TLC is much preferred over HPLC. When compared with HPLC, TLC is much less expensive, easier to perform, uses simple equipment, uses much less sample, and the detection of the carbohydrates directly on the plate is more sensitive. TLC is also more reproducibile. With HPLC, once a sample has been added to a column, the column is irreversibly modified. Because of the high cost of the HPLC columns, many hundreds of samples must be separated and analysed in series on the same column to make the procedure economically feasible; whereas sample placed on to a TLC plate is separated in its own little column or lane, which is not affected by other compounds that have previously been adsorbed and separated on the support material. Further, approximately 18 samples can be simultaneously separated on a single 20×20 cm TLC plate, which can include standards. A simple comparison of the migration positions with the migration positions of the standards can be used for qualitative identification.

TLC Support Material, Solvents and Detection Methods

The principal support material found most useful for valuable in the separation of carbohydrates is silica gel, a stable and inert substance. The silica gel thin layer is usually uniformly 250 μm thick over the TLC plate. The resolution of carbohydrates is achieved by the selection of a specific solvent system. Many solvent systems have been empirically developed. The particular solvent used depends on whether the carbohydrates are monosaccharides, disaccharides, trisaccharides or oligosaccharides, or whether they contain a charged group such as a uronic acid, an amino sugar or a carbohydrate phosphate.

A relatively simple solvent that has found valuable use in the separation of a number of mono-, di- and trisaccharides is acetonitrile–water (85 : 15, v/v). This solvent is frequently the first choice when separating unknown carbohydrates in a sample. Solvents for the separation of more complex mixtures can then be developed using other organic compounds in combination with the acetonitrile–water system. Many solvent systems contain three components: water, a water-soluble component and a water-insoluble component. The three components are combined in various proportions so as to give a homogeneous solution. For maximum resolution, the technique of multiple ascents is frequently employed. In this technique, the solvent is allowed to ascend to the top of the TLC plate, whereupon the plate is removed from the developing tank and the solvent is completely evaporated from the plate. The plate is then put back into the TLC tank for a second, third and/or fourth ascent. **Figure 1** illustrates the separation of relatively complex mixtures of carbohydrates using this technique with acetonitrile–water (85 : 15, v/v) solvent.

A very sensitive detection system has recently been developed in which most carbohydrates can be detected in the 50–2000 ng range, directly on the TLC plate. The method uses a detecting reagent, consisting of 0.3% (w/v) of *N*-(1-naphthyl)ethylenediamine and 5% (v/v) concentrated sulfuric acid in methanol. The TLC plate is removed from the developing tank, dried and rapidly dipped into the reagent. The plate is dried and placed in an oven at 120°C for 10 min. Carbohydrates give blue-black spots on a white background. Most carbohydrates can be detected by using this reagent, with the exception of 2-amino-2-deoxy sugars, 2-*N*-acetylamino-2-deoxy sugars and sugar alcohols. This is primarily due to the requirement of forming furfurals from the carbohydrates by dehydration with the sulfuric acid. An older method used sulfuric acid charring, in which a 25% (v/v) solution of sulfuric acid in methanol was sprayed on to the TLC plate, dried and then heated at 120°C for 10 min. This is a much less sensitive method, with detection in the 10–100 μg range. Further, the

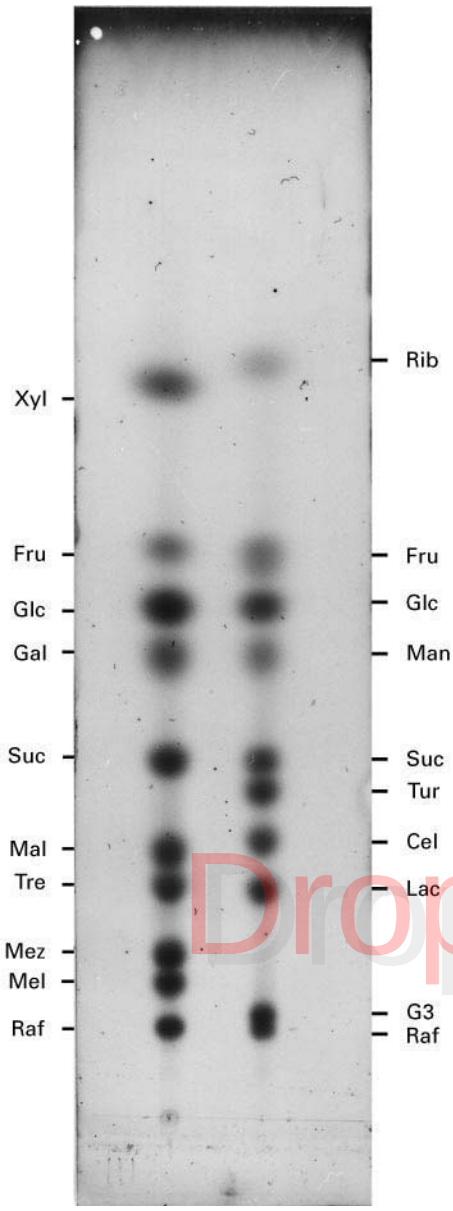

Figure 1 Separation of various mono-, di- and trisaccharides on Whatman K5 TLC plates (18.5 cm solvent path length for each ascent), using four ascents of acetonitrile–water in volume proportions of 85 : 15 at 20–22°C. The carbohydrates were detected by using the *N*-(1-naphthyl)ethylenediamine–sulfuric acid–methanol dipping reagent.

spraying technique is much less environmentally friendly than a dipping technique.

The separation of radiolabelled carbohydrates permits several sensitive techniques to be used to detect and quantify the carbohydrates directly on the TLC plate, such as a TLC radioactive scanner or a PhosphoImager. Both can be used to detect and quantitate ^{14}C, ^{35}S, or ^{32}P radioactive isotopes, although the PhosphoImager is several orders of magnitude more sensitive and relatively easy to use quantitatively.

TLC Separation of Homologous Series of Oligosaccharides

The separation of oligosaccharides is important in a wide variety of applications, such as analysis of carbohydrates in foods, analysis of oligosaccharides from glycoproteins and glycolipids, the analysis of enzymatic synthesis of oligosaccharides and the analysis of the hydrolysis of polysaccharides. Many of these analyses involve the separation of a homologous series of oligosaccharides containing one or possibly two kinds of glycosidic linkages and as many as 20–30 monosaccharide residues of the same type in the oligosaccharides. The separation of a homologous series of saccharides, containing 2–30 monosaccharides residues, linked β-1 → 2, β-1 → 3, α-1 → 4, and α-1 → 6, has been reported, using silica-gel plates developed with butanol-1–ethanol–water. The development time was however, several hours for one ascent.

Much faster systems, using Whatman K5 plates or Merck silica gel 60 plates and multiple ascents with acetonitrile–water systems, have been developed for the separation of maltodextrins (α-1 → 4 linked glucosaccharides) and isomaltodextrins (α-1 → 6 linked glucosaccharides). The separation of maltodextrins was achieved using acetonitrile–ethyl acetate–propanol-1–water in volume proportions of 85 : 20 : 50 : *x*. The amount of water (*x*) can be varied from 50 to 70 parts by volume, depending on the desired number of saccharides to be separated. The largest saccharides can have 20 D-glucose residues. Likewise, using up to four ascents can increase the number of saccharides separated. **Figure 2** illustrates the separation of maltodextrins using one to four ascents of 85 : 20 : 50 : 60 volume proportions at 20–22°C.

Isomaltodextrins migrate more slowly than an equivalent maltodextrin mixture, primarily because of differences caused by the α-1 → 6 glycosidic linkage vs the α-1 → 4 glycosidic linkage. To obtain an equivalent separation for the isomaltodextrins, the water content (*x*) of the solvent system has to be increased to 90 or 100 volume proportions. **Figure 3** illustrates the separation of isomaltodextrins using one to four ascents of acetonitrile–ethyl acetate–propanol-1–water in the volume proportions of 85 : 20 : 50 : 90 at 20–22°C.

The power of TLC separations of saccharides, using these solvents, is illustrated in **Figure 4** in which complex saccharides are separated from mixtures produced by the action of two different enzymes

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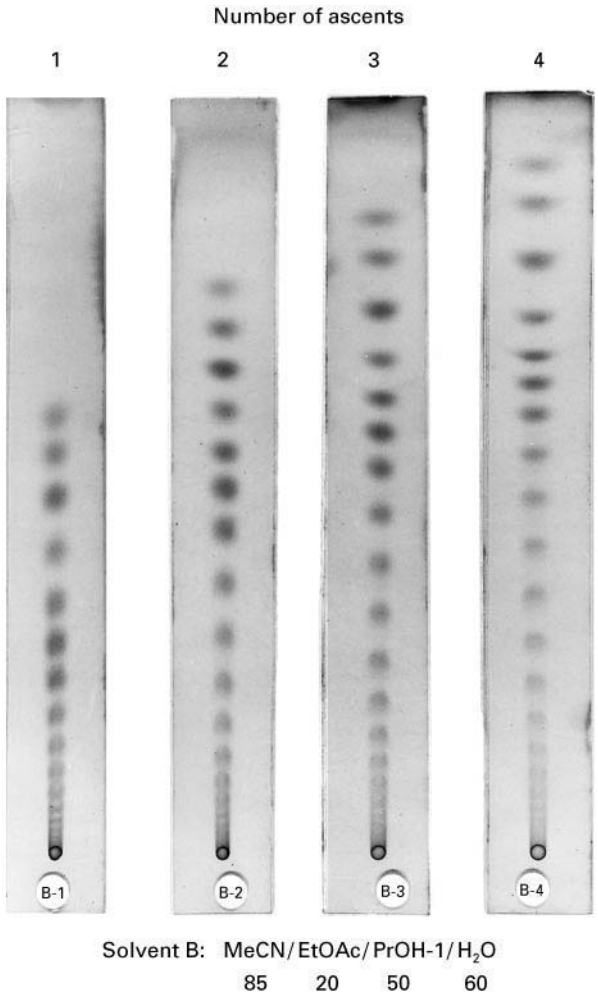

Figure 2 Separation of D-glucose and maltodextrins on Whatman K5 TLC plates (18.5 cm solvent path length for each ascent), using 1–4 ascents of acetonitrile–ethyl acetate–propanol-1–water in volume proportions of 85 : 20 : 50 : 60 at 20–22°C. The numbers at the top of each chromatogram indicate the number of solvent ascents. The carbohydrates were detected using the *N*-(1-naphthyl)ethylenediamine–sulfuric acid–methanol dipping reagent. Reproduced with permission from Robyt and Mukerjea (1994).

acting on polysaccharides. The saccharides were separated by using four ascents of acetonitrile–ethyl acetate–propanol-1–water in volume proportions of 85 : 20 : 50 : 50 at 20–22°C on a Whatman K-5 plate. Lane 1 on the plate is a set of maltodextrin standards, containing D-glucose, maltose, maltotriose and so forth down to maltodextrins with 14 D-glucose residues; lane 2 shows an equivalent set of maltodextrin saccharides that were obtained by the action of isoamylase acting on shellfish glycogen; lane 3 shows the separation of products that resulted from the action of porcine pancreatic α-amylase acting on potato amylopectin. Saccharides marked B6, B7 and B8 contain six, seven and eight D-glucose residues, respectively having one α-1 → 6 glycosidic linkage and five, six and seven α-1 → 4 glycosidic linkages. These saccharides migrate in between the maltodextrins, for example, BB6 migrates behind G6 and faster than G7, B7 migrates behind G7 and faster than G8, and so forth. Saccharides marked BB9, BB10 and BB11 contain nine, 10 and 11 D-glucose residues, respectively, with each having two α-1 → 6 glycosidic linkages and seven, eight and nine α-1 → 4 glycosidic linkages. Saccharides containing one D-galactose residue will usually migrate more slowly than an equivalent saccharide containing all D-glucose

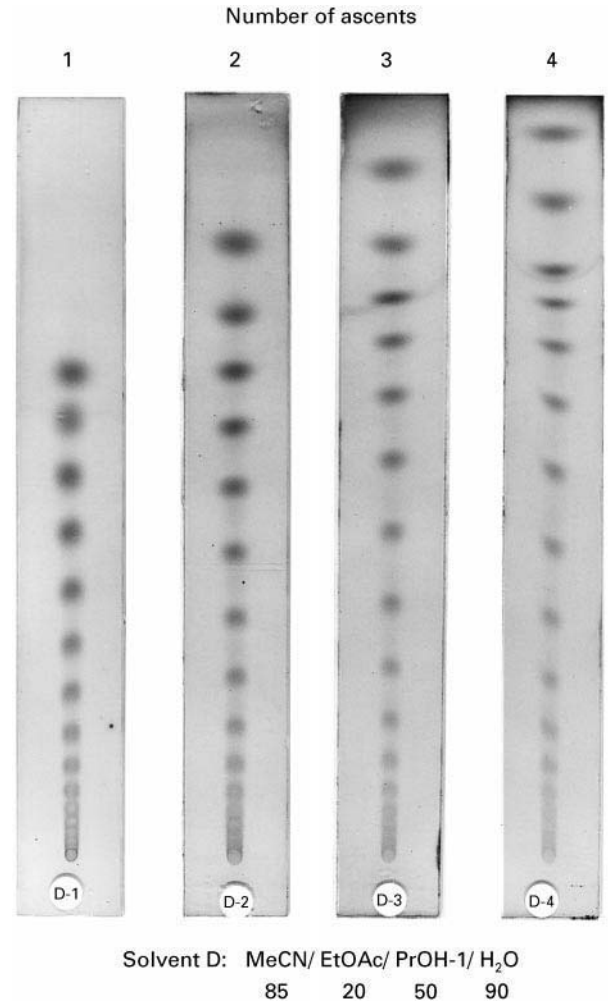

Figure 3 Separation of D-glucose and isomaltodextrins on Whatman K5 TLC plates (18.5 cm solvent path length for each ascent), using 1–4 ascents of acetonitrile–ethyl acetate–propanol-1–water in volume proportions of 85 : 20 : 50 : 90. The numbers at the top of each chromatogram indicate the number of solvent ascents. The carbohydrates were detected using *N*-(1-naphthyl)ethylenediamine–sulfuric acid–methanol dipping reagent. Reproduced with permission from Robyt and Mukerjea (1994).

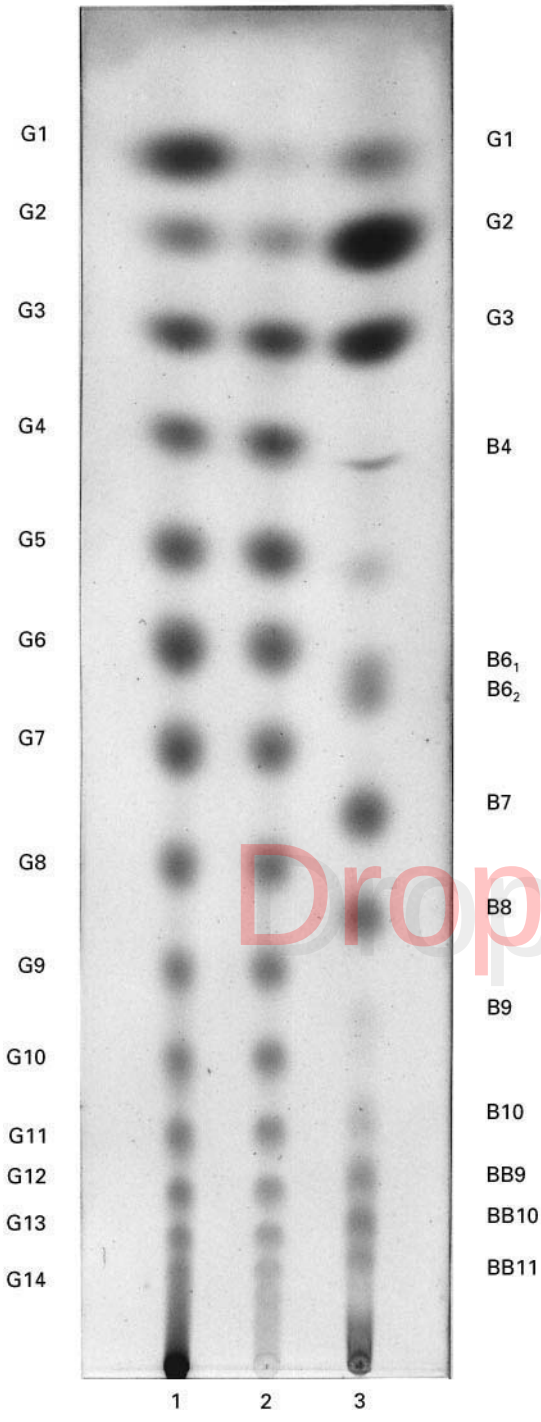

Figure 4 Separation of maltodextrins and maltodextrins, containing one and two α-1 → 6 linkages. Lane 1, glucose and maltodextrin standards; lane 2, maltodextrins resulting from the action of isoamylase reaction with shellfish glycogen; lane 3, α-amylase action on amylopectin. Whatman K5 TLC plate (18.5 cm solvent path length for each ascent) was irrigated four times with acetonitrile–ethyl acetate–propanol-1–water in volume proportions of 85 : 20 : 50 : 50 at 20–22°C. The carbohydrates were detected using the *N*-(1-naphthyl)ethylenediamine–sulfuric acid–methanol dipping reagent. Reproduced with permission from Robyt and Mukerjea (1994).

residues. Compare the migration of lactose with cellobiose in Figure 1.

To illustrate the tremendous power that TLC has in separating carbohydrate oligosaccharides with very similar structures, **Figure 5**A shows the chromatographic separation of saccharides formed by the action of *Leuconostoc mesenteroides* B-512FM dextransucrase transfer of D-glucose from sucrose to malto-oligosaccharide acceptors having two to eight D-glucose residues. Two different products were formed by the transfer of D-glucose to the reducing end of the acceptor saccharide to form an α-1 → 6 linkage (product-1a) and transfer of D-glucose to the nonreducing end of the acceptor saccharide to form α-1 → 6 linkage (product-1b). Products 2a, 2b, 3a, 3b and so forth are products resulting from the transfer to C_6 of reducing-end glucose residue and to C_6 of the nonreducing-end glucose residue, and so forth of the first, second and third acceptor products. These complex products were separated using four ascents of ethyl acetate–ethanol–water (2 : 2 : 1, v/v/v) on a Whatman K5 TLC plate.

Figure 5B shows the separation of an even more complex mixture of products, containing the same number of D-glucose residues, but differing from each other by the location of the transferred D-glucose residue and the types of linkages, either α-1 → 6 or α-1 → 3. D-Glucose was transferred from sucrose to maltotriose by the action of three enzymes (F, I and S). Enzyme I gave four initial products, 1a–1d; 1a had D-glucose transferred to C_6 of the reducing-end glucose residue of maltotriose; 1b had D-glucose transferred to C_6 of the nonreducing-end glucose residue of maltotriose; 1c had D-glucose transferred to C_3 of the reducing-end glucose residue of maltotriose; and 1d had D-glucose transferred to C_3 of the nonreducing-end glucose residue of maltotriose. Enzymes F and S only gave two initial products, 1a and 1b. All three enzymes gave higher products: 2a, 2b, 3a, and so forth.

Separation and Detection of Sugar Alcohols on TLC

One of the more difficult separations of carbohydrates is that of separating aldoses from their corresponding alditols (sugar alcohols). Alditols can be separated from their aldoses on TLC by using two ascents of acetonitrile–ethyl acetate–propanol-1–water in volume proportions of 85 : 20 : 20 : 15. As previously mentioned, alditols are not readily detected by the sulfuric acid-based reagents described above. The best detection system is an alkaline silver nitrate–sodium thiosulfate dipping system that will detect alditols in the range of 500 ng to 1 μg. The dry TLC plate is dipped into a silver nitrate–acetone

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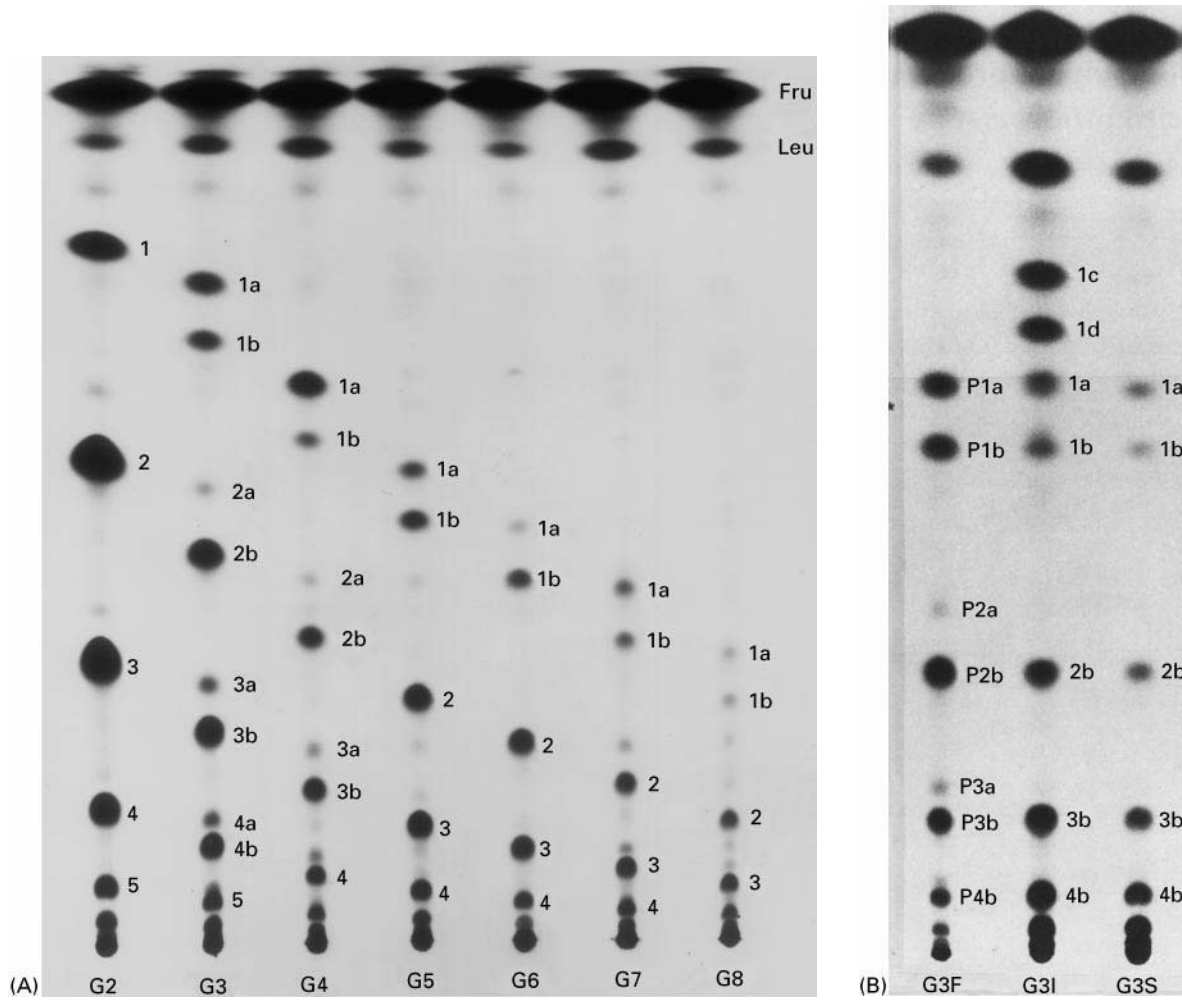

Figure 5 (A) TLC separation of a series of products produced by the action of *Leuconostoc mesenteroides* B-512FM dextransucrase catalysed transfer of D-glucose from sucrose to maltodextrin acceptors, maltose (G2) to maltooctaose (G8). With G3 and higher, the enzyme gave two products of the same size, transferring D-glucose to the C_6 position of the reducing-end glucose residue of the acceptor (product 1a) and the transfer of D-glucose to C_6 position of the nonreducing-end glucose residue of the acceptor (product 1b). Products 2a, 2b, 3a, 3b and so forth represent transfer to C_6 position of the reducing-end and the nonreducing-end glucose residues of the first, second, and so forth acceptor products. The saccharides were separated on Whatman K5 TLC plates using four ascents of ethyl acetate–ethanol–water in volume proportions of 2 : 2 : 1 (18.5 cm solvent path length for each ascent). The compounds were labelled with ^{14}C and detected by autoradiography. Reproduced with permission from Fu and Robyt (1990). (B) Separation of the products from the transfer of D-glucose from sucrose to maltotriose by the action of three enzymes, *Leuconostoc mesenteroides* B-512FM dextransucrase (F), *Streptococcus mutans* mutansucrase (I), and *S. mutans* dextransucrase (S). Whatman K5 TLC plate was irrigated with four ascents of ethyl acetate–ethanol–water, in volume proportions of 2 : 2 : 1. The compounds were labelled with ^{14}C and detected by autoradiography. Reproduced with permission from Fu and Robyt (1991).

solution for 5 min. The reagent is prepared by adding 1 mL of saturated aqueous silver nitrate to 200 mL of acetone, followed by the dropwise addition of distilled water until the silver nitrate is dissolved. The plate is dried and then dipped into an alkaline methanol solution containing 0.4% NaOH (w/v) for 30 min. Brown to black spots appear for the alditols. The plate is then rapidly dipped into a 1.5 mol L^{-1} solution of $Na_2S_2O_3$ containing 0.08 mol L^{-1} Na_2SO_3 and 0.25 mol L^{-1} $NaHSO_3$, and then the plate is washed for 1 min in running water, giving black spots on a white background. **Figure 6** illustrates the separation of seven different alditols from their corresponding aldoses.

Separation of Methylated Monosaccharides by TLC

The separation of *O*-methylated monosaccharides is especially important. *O*-Methylated monosaccharides are produced from the methylation of oligosaccharides and polysaccharides followed by

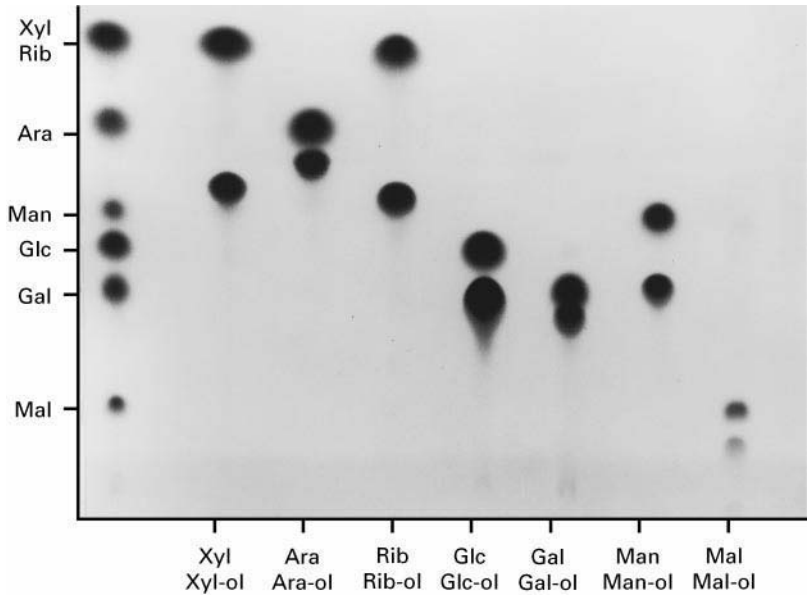

Figure 6 Separation of alditols (sugar alcohols) from their corresponding aldoses on Whatman K5 TLC plates (18.5 cm solvent path length for each ascent) using two ascents of acetonitrile–ethyl acetate–propanol-1–water in volume proportions of 85 : 20 : 20 : 15 at 20–22°C. The carbohydrates were detected by using the alkaline silver nitrate dipping reagents. Reproduced with permission from Han and Robyt (1998).

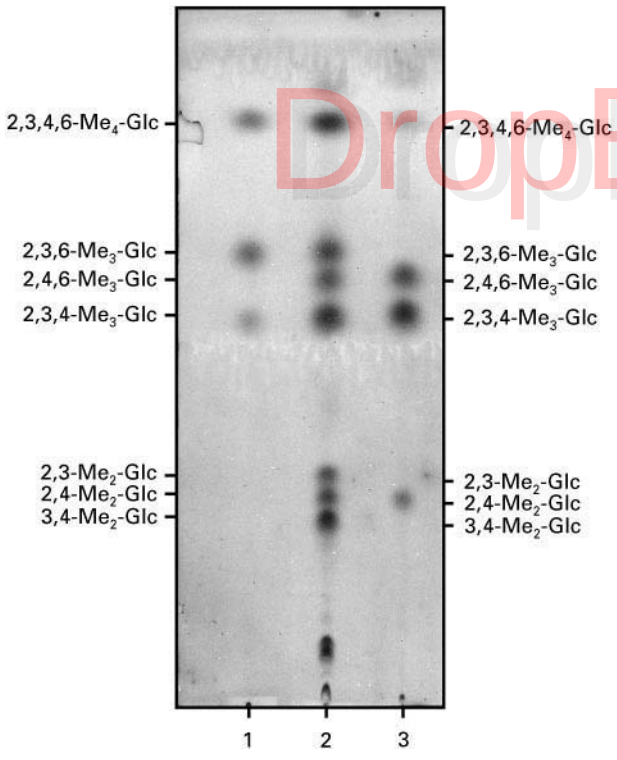

Figure 7 Separation of *O*-methylated D-glucoses. Lanes 1 and 3 are standards; lane 2 is the analysis of a complex, highly branched D-glucan. Whatman K6 plates were irrigated (18.5 cm solvent path length for each ascent) at 20–22°C, using two ascents of acetonitrile–chloroform–methanol in volume proportions of 3 : 9 : 1. The carbohydrates were detected by using the *N*-(1-naphthyl)ethylenediamine–sulfuric acid–methanol dipping reagent. Reproduced with permission from Mukerjea *et al.* (1996).

acid hydrolysis. The number of methyl groups and their location on the resulting monosaccharides indicate the positions of the linkages to the monosaccharides in the oligosaccharides or polysaccharides and whether or not the saccharides are branched. For example, if a gluco-polysaccharide contains $1 \rightarrow 4$ glycosidic linkages with $1 \rightarrow 6$ branch linkages, there would be three kinds of methylated monosaccharides: 2,3,6-tri-*O*-methyl-D-glucose (major), 2,3,4,6-tetra-*O*-methyl-D-glucose (minor from the nonreducing-end D-glucose residues) and 2,3-di-*O*-methyl-D-glucose (minor from the branched D-glucose residues). The use of TLC to separate and analyse these methylated monosaccharides has greatly simplified methylation analysis of oligo- and polysaccharides. Further, because of the sensitivity of detecting the methylated monosaccharides (lower limits of 25–50 ng range) directly on the TLC plate, micro amounts of saccharide (10 μg) can be methylated and analysed. **Figure 7** illustrates the chromatography of methylated D-glucoses on Whatman K6 TLC plates, using two ascents of acetonitrile–chloroform–methanol in volume proportions of 3 : 9 : 1 at 20–22°C. The methylated carbohydrates are detected by dipping the plate into the *N*-(1-naphthyl) ethylenediamine–sulfuric acid–methanol reagent described above, followed by heating for 10 min at 120°C.

Quantitative Determination of Carbohydrates

The easiest method of detecting and quantitating carbohydrates on a TLC plate is to use ^{14}C-labelled

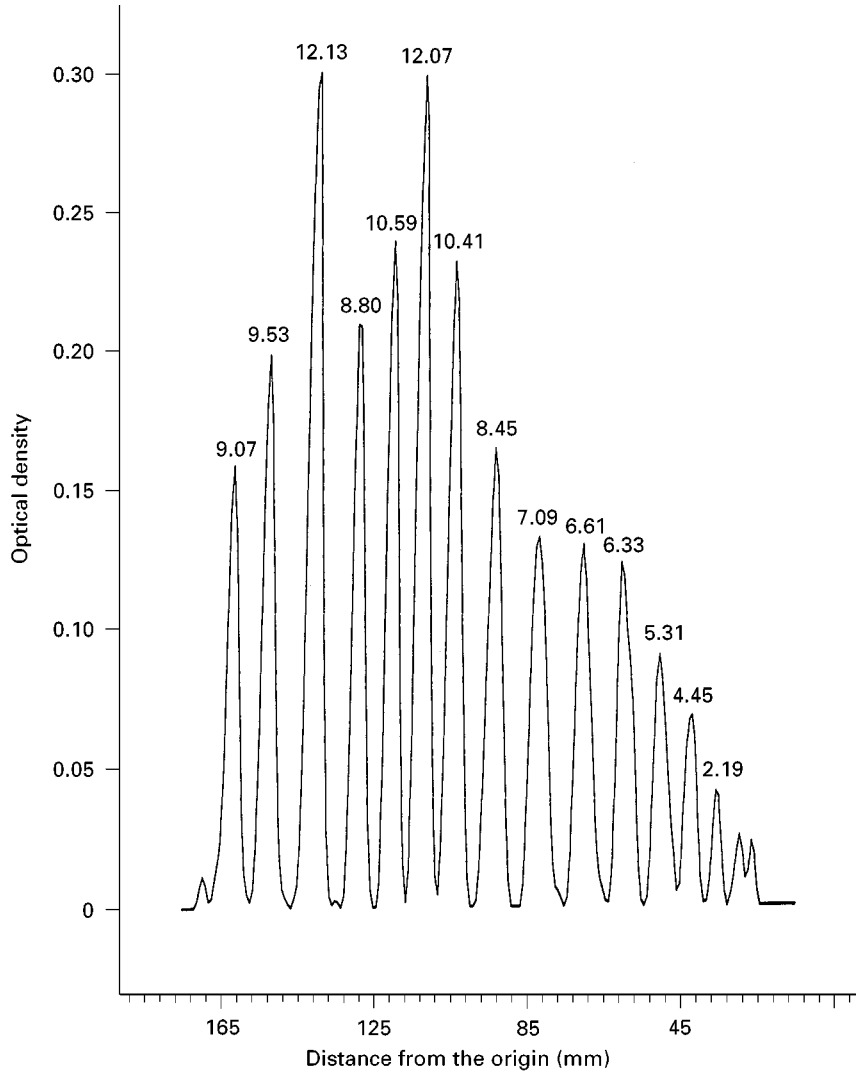

Figure 8 Densitometric scan of TLC number 3 shown in Figure 2, using a Bio-Rad imaging densitometer. The relative weight per cents for each of the carbohydrates are shown at the top of each peak. The first peak is D-glucose, followed by maltodextrins: maltose, maltotriose, maltotetraose, and so forth, down to a maltodextrin with 14 D-glucose residues.

carbohydrates. The radioactivity can be detected and quantitated directly on the plate using a phosphoimager. The use of radioactively labelled carbohydrates, however, is limited to carbohydrates that can be obtained in radioactive form and therefore limited in the kinds of experiments that can be performed. The more usual case is the chromatography of nonlabelled carbohydrates. The detection of carbohydrates on a TLC plate, using a sulfuric acid-based reagent, such as *N*-(1-naphthyl)ethylenediamine reagent described above, can be quantified using a scanning densitometer. Absolute, quantitative amounts can be determined using various amounts of the monosaccharide involved in the saccharides as standards, for example, D-glucose, D-mannose, or sucrose, and so forth. However, for many purposes, the relative amounts of the individual saccharides in a mixture are sufficient. In this instance, the individual saccharides that are separated in a mixture can be scanned, the densities of the compounds summed and the percentage of the individual compounds computed by dividing the individual densities by the sum of the densities. **Figure 8** shows a density scan of chromatogram no. 3 in **Figure 2**.

Preparative Separation of Carbohydrates Using TLC

By using the same solvent systems as is used in analytical TLC separations, but substituting TLC plates

that have a much thicker silica gel layer (1000–2000 μm), preparative separations of carbohydrates can be obtained. Approximately 25–50 μL of a 20–30 mg sample of a mixture of carbohydrates is streaked along a sample application line, 15 mm above the bottom of a 20 × 20 cm TLC plate. The plate is developed in the usual way. After development, 15 mm strips are cut off from each end of the chromatogram and dipped into the detecting reagent. The strips are lined up with the plate and the locations of the desired compounds are marked. The silica gel area containing the compound is scraped from the plate into approximately 2 mL of water. The suspension is mixed and the silica gel removed by filtration or centrifugation. The aqueous extract can then be concentrated to dryness by evaporation, and the compound redissolved if necessary.

Points to Consider to Obtain Good Separations

1. A relatively small (1–25 μL) measured amount of sample should be carefully added on to the TLC plate, keeping the size of the spot as small as possible. The use of a microlitre syringe pipette, such as a Hamilton syringe, is desirable. When many samples are to be added to the TLC plate, a multi-spotting instrument can conveniently be used to make very small, uniform spots with a minimum of effort. The multi-spotter is also convenient when a relatively large volume, for example 10–25 μL, of sample is to be added.
2. Be sure that the developing tank is tightly sealed during development (vacuum grease can be used with glass-to-glass surfaces). The solvent and vapour should be in equilibrium before beginning the development.
3. Allow the solvent to ascend to the *top* of the TLC plate to give the maximum path length for the solvent. The old idea that the TLC plate should be removed from the solvent *before* the solvent has reached the top or *just as* the solvent reaches the top of the plate is not valid, if the chamber is tightly sealed. In fact, it is best to allow 5–10 min extra, after the solvent reaches the top of the TLC plate, before removing the plate. This ensures that the plate has been completely saturated with the solvent over the entire surface of the plate.
4. Use no more than 15 mm margin from the bottom of the plate to the point of sample application, so as to give the maximum path length for the solvent. Also, be sure only to use pencil to mark the TLC plates.
5. In multiple ascending chromatography, dry the plates thoroughly between each ascent. The use of a hair-dryer is a fast and convenient method for drying plates.
6. In developing the chromatogram, rapidly and carefully dip the irrigated plate into the detecting reagent to obtain a uniform amount of reagent over the entire plate. The use of a narrow 1.5 × 22 × 22 cm glass chamber is convenient for dipping. A stand and a lid have to be fabricated from polypropylene or similar material to hold the chamber (**Figure 9**).

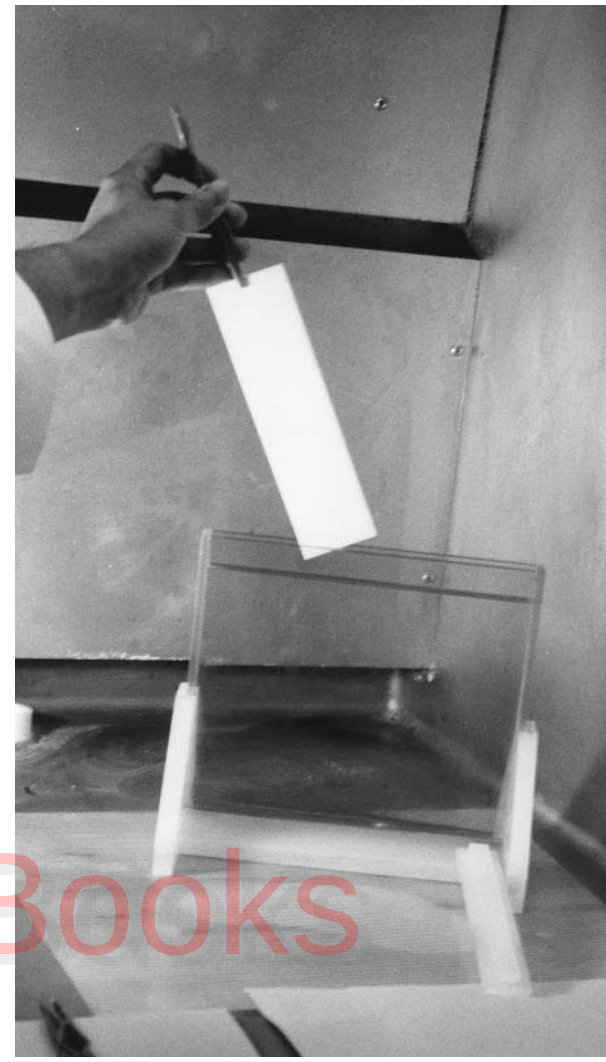

Figure 9 Glass chamber and holder used to dip the TLC plates into the detecting reagent.

Conclusions and Future Prospects

TLC has become a powerful analytical tool for separating compounds with very similar chemical and

physical properties, such as carbohydrates. The development of reliable and reproducible commercial TLC plates has placed it at the forefront of separation methods that can be used for both qualitative and quantitative determinations. Further, the use of relatively simple and inexpensive materials permits a wide range of laboratories, from the high-school teaching laboratory to quality control and research laboratories, to analyse and compare compounds easily, quickly and with high sensitivity. The use of relatively inert materials, such as silica gel on glass, allows chemical reactions to be conducted directly on the plate where the compounds have been separated. These reactions provide qualitative identification of the compounds by specific colour production and the quantitative determination of the compounds by the use of scanning densitometry. The latter is particularly important for carbohydrates as they do not lend themselves to the usual physical detection and quantifying methods, such as ultraviolet or visible absorbance, light scattering or refractive index. The development of a wide variety of solvents has also permitted the separation of carbohydrates that differ from each other in subtle aspects of configuration of chiral centres, differences in the kinds of glycosidic linkages present, and differences in the size of the compounds.

Future developments in TLC of carbohydrates may come slowly as much progress has been made in the last 50 years. But, as in most areas of endeavour, need is the necessity of invention. Developments will undoubtedly be made in new solvents that will separate new kinds of mixtures of carbohydrates obtained from natural products and from chemical and enzymatic modification reactions. There will probably be detection methods developed that will give greater sensitivity in the submicro range of carbohydrates on the plate. The latter will then also provide for the development of new physical instrumentation using lasers for scanning density, reflectance and fluorescence in quantifying carbohydrates directly on the plate.

See also: **II / Chromatography: Thin-Layer (Planar):** Layers; Preparative Thin-Layer (Planar) Chromatography; Radioactivity Detection; Spray Reagents. **III / Carbohydrates:** Electrophoresis; Gas Chromatography and Gas Chromatography-Mass Spectrometry; Liquid Chromatography.

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